

Cryptococcosis

A Cause of Calcified Intracranial Mass Lesions

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CRYPTOCOCCUS NEOFORMANS is the most common fungal infection of the central nervous system. Clinical manifestations of this infection are protean and may simulate primary psychiatric illness, tuberculous meningitis, sarcoidosis of the central nervous system or brain tumor. Chronic meningitis is the usual manifestation of central nervous system cryptococcosis. Occasionally granulomata may occur which are large enough to act as intracranial mass lesions. We had the opportunity to observe a patient with multiple intracerebral cryptococcomas in whom negative india

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ink preparations and fungal cultures of spinal fluid obscured the cause of illness. Other striking features of his cryptococcal infection were the development of intracerebral calcifications and positive radioisotope brain scans.

Report of a Case

In July 1970 a 47-year-old white man was admitted to hospital in Honolulu, with complaint of urinary incontinence and weakness of the left arm and leg. During the preceding 16 months he had received psychiatric treatment for alcoholism and depression. He had a history of rheumatic heart disease without symptoms of cardiac decompensation.

On physical examination he had murmurs of aortic stenosis and insufficiency, hepatomegaly, psoriasis, and mild left hemiparesis. An x-ray film of the chest showed a 2.5 centimeter coin lesion in the left mid-lung field which had not been present on a film taken in January 1969; this was presumed to be a pulmonary infarct. Skull films were normal. Cerebrospinal fluid examination (Table 1) showed increased protein, normal sugar, and slight pleocytosis. Routine culture and an india ink stain of the fluid were negative. A brain scan showed multiple areas of increased isotope uptake.

The patient improved on supportive care and antibiotics and was discharged in August 1970 with diagnoses of chronic alcoholic encephalopathy, multiple cerebral infarcts (although cerebral carcinomatosis was also considered), left lung infarct and rheumatic heart disease with aortic stenosis and aortic insufficiency.

Subsequently, he had two episodes of weakness

TABLE 1—Results of Examination of Spinal Fluid

Date	Opening Pressure mm/Water	White Cell Count/mm ³	Mono- nuclear	Protein Mg%	Glucose Mg%	Routine Culture	India Ink	Fungal Culture	AFB Culture
8/10/70	150	7	5	116	61	—	—
12/ 9/70	320	41	36	93	63	Erwinia	—	—	—
12/14/70	..	50	48	—	..	—	—
2/ 5/71	225	14	12	77	50	—
7/ 8/71 (PEG)	..	5	2	48	50
7/19/71 (Ventricular)	..	4	4	19	75
8/ 4/71	320	62	59	60	48	—
8/21/71	320	197	167	82	20	—	—	—	—
8/27/71	190	8	6	114	65	..	—	..	—
9/24/71	395	30	28	45	33	—	..	—	—
10/ 8/71	360	37	11	115	45
10/15/71	400	580	116	90	40	—	..	—	..
10/22/71 (Ventricular)	..	1	0	16	72	—
11/11/71	240	41	41	60	48	—

—=Negative

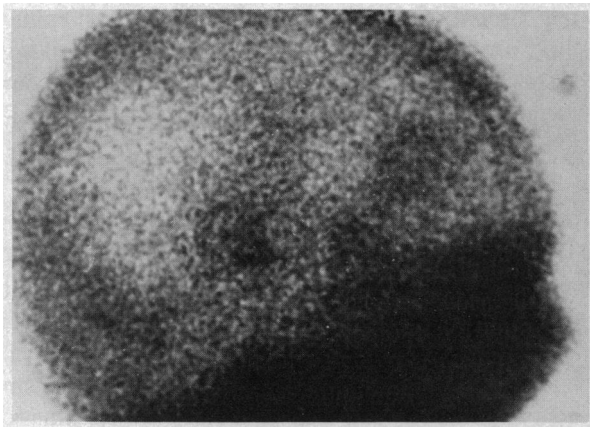


Figure 1.—Brain scan, showing multiple abnormal foci of increased isotope uptake.

and clonic movements of the right upper extremity. A brain scan in October 1970 again showed multiple abnormal foci. The patient was readmitted to hospital in November 1970. Examination revealed impaired mentation, increased deep tendon reflexes in the left extremities and ataxic movements of the left leg. Cerebrospinal fluid (Table 1) showed moderate mononuclear pleocytosis, increased protein and normal glucose. In addition, a Gram-negative rod was cultured from the fluid; it was later identified as an *Erwinia* species.

The patient was thought to have either vasculitis or multiple septic emboli from rheumatic valvulitis. Multiple blood cultures were negative. Administration of tetracycline, prednisone, isoniazid and diphenylhydantoin was begun. On this regimen his mental condition gradually improved and he was discharged in January 1971. At this time the patient and his family moved from Hawaii to California. He continued to take prednisone, 20 mg a day, isoniazid, and penicillin V.

On 6 June 1971 the patient had a major motor seizure and was admitted to Kaiser Hospital. Babinski's sign on the left was noted postictally, but results of neurological examination the following day were within normal limits. An electroencephalogram showed a focus of 2 to 4 cycles per second slow activity in the right temporal region with a smaller amount of slowing in the left temporal area. A technetium⁹⁹ brain scan demonstrated increased uptake in the right posterior temporal, left parietal and right frontal areas (Figure 1). Skull films (Figure 2) now showed multiple intracranial calcifications of varying size ranging from 0.5 to 0.75 cm in diameter.

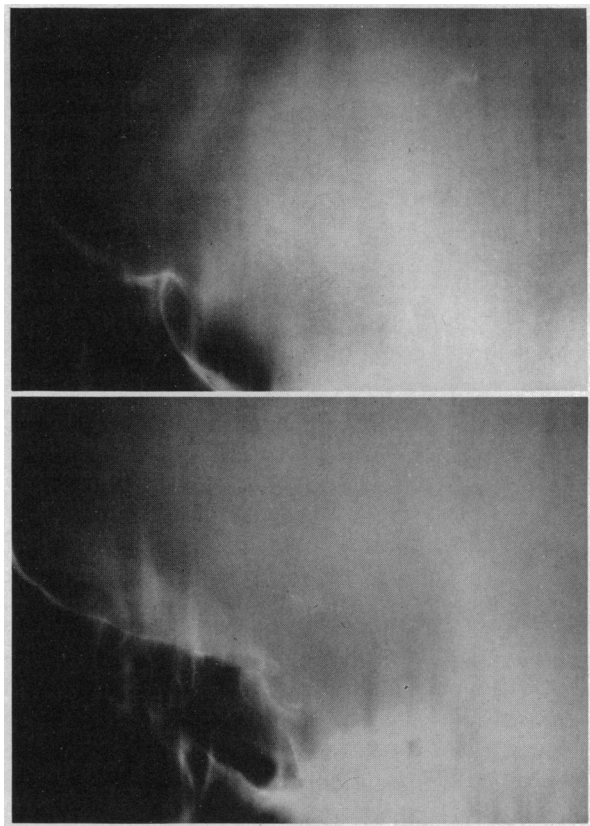


Figure 2.—Laminograms of skull showing intracerebral calcifications in the left parietal region and in the right temporal area just superior to the right anterior clinoid process of the sella turcica.

Bilateral carotid angiograms showed stretching of the thalamo striate veins bilaterally, suggesting moderate enlargement of the lateral ventricles. Pneumonencephalography showed normal subarachnoid cisterns and cerebral sulci, but the ventricular system could not be filled. A ventriculogram on 19 July 1971 showed dilated lateral ventricles without evidence of ventricular obstruction. However, indentations into the ventricular system suggested multiple small mass lesions.

In late August 1971, the patient complained of severe headaches. Examination showed mild nuchal rigidity, confusion, nystagmus on lateral gaze to either side, Babinski's sign on the left and ataxic movements of the left arm and leg. On lumbar puncture (Table 1) the fluid showed a pronounced increase in the number of white cells and for the first time an abnormally low glucose value. The prednisone dosage was tapered beginning on 26 August 1971 and was finally discontinued 5 September 1971. Previous attempts to reduce corticosteroid therapy had always resulted in mental deterioration.

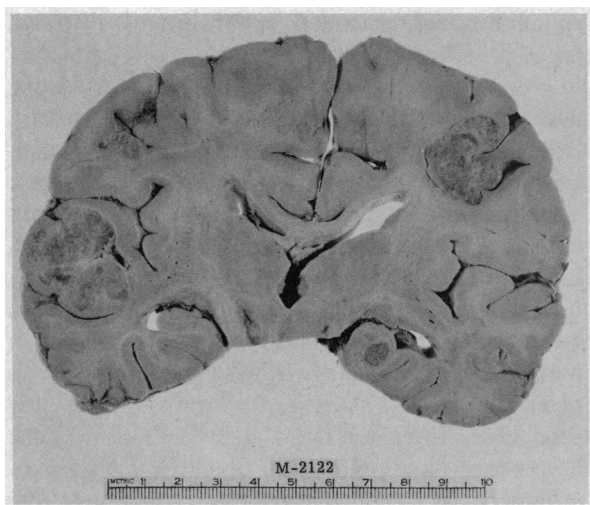


Figure 3.—Coronal section at the level of the red nucleus showing multiple granulomas. Small granulomatous lesions are seen in the right parasagittal region and in the right substantia nigra.

At diagnostic craniotomy performed 2 September 1971, a yellow, firm 2×1.5 cm mass was removed from the medial tip of the right frontal lobe. India ink mounts and permanent sections from this tissue revealed multiple encapsulated yeasts, typical of *Cryptococcus neoformans*. Culture of the excised tissue was subsequently positive for the fungus. Intravenous administration of amphotericin B was begun. On 10 September 1971, results of cryptococcal antigen titers by latex agglutination were reported to be 1:8 in spinal fluid and 1:4 in serum.* Cryptococcal antibody titers were negative.

On 20 September 1971, after a cumulative intravenous dose of 690 mg of amphotericin B, the patient began to have grand mal seizures. Babinski's on both sides, increasing confusion and ataxia were noted. Ventriculograms showed a mass in the inferior portion of the left cerebellar hemisphere. A second craniotomy was performed and a 2.5×1.5 cm cryptococcal granuloma was excised from the left cerebellum. Cerebrospinal fluid cryptococcal antigen titer was 1:2 on 4 November 1971, by which time the patient had received 1,730 mg of amphotericin B intravenously. He died suddenly on 24 November 1971.

Autopsy

The brain showed multiple firm tan lesions varying from 0.3 to 3.5 cm in diameter (Figures 3 and 4). These lesions, some of which were grossly gelatinous, were found in the cerebral

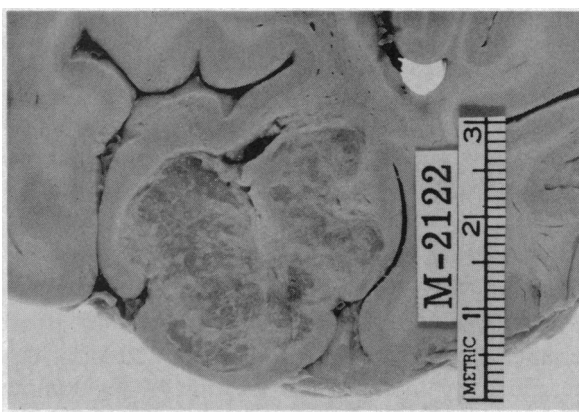


Figure 4.—Detail of granuloma, showing sharp circumscription, grossly visible septations and gelatinous appearance of intervening areas.

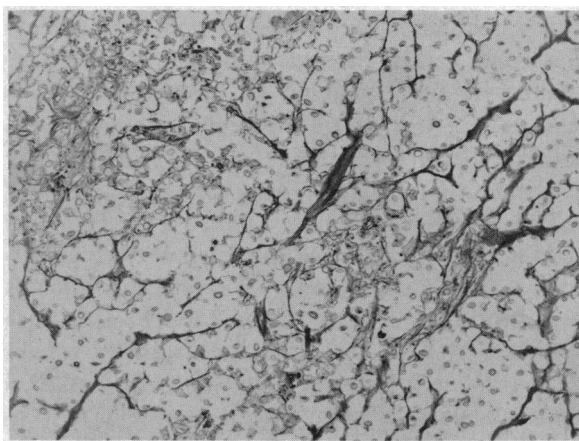


Figure 5.—Section of one granuloma, showing fibrous connective tissue septae and numerous organisms.

hemispheres, cerebellum and pons. Microscopically they were identified as granulomata with minimal to moderate amounts of fibrous connective tissue within the walls and with abundant chronic inflammatory response. Giant cells were seen only rarely. Within the granulomas were innumerable cryptococcus organisms (Figure 5). In the adjacent brain tissue there was mild to moderate gliosis and demyelination. There were several other torulomas in the meninges surrounding the pituitary gland. No evidence of cryptococcal infection was found in other organs. Severe fibrous thickening with dystrophic calcification of the aortic valve, consistent with healed rheumatic valvulitis was observed. There was no evidence of bacterial vegetations. The lungs were unremarkable except for the changes of apical pulmonary emphysema. The liver was enlarged and microscopic examination showed moderately extensive portal cirrhosis.

*Performed at the Center for Disease Control, Atlanta, Georgia.

Discussion

Cryptococcosis almost always presents clinically as meningitis. However, unusual forms of this disease with granulomas of the brain or spinal cord are occasionally seen.¹ Vijayan et al² discovered only 23 confirmed cases in a review of the literature and personally studied one more. Three of the 24 patients were totally asymptomatic during life. In the symptomatic cases the usual clinical presentation was that of an intracranial mass lesion with symptoms and signs determined by the size and location of the tumor. Symptoms preceded hospital admission by from two weeks to 12 years. In 15 of the 20 symptomatic cases the lesion was single and isolated. The cerebrospinal fluid was entirely normal in four cases. Increased protein, lymphocytic pleocytosis and decreased sugar were common abnormalities in the remaining 16 cases.

Reports of calcification within intracranial torulomas are even more rare. Neuhauser and Tucker³ described multiple, generally punctate areas of calcification on skull x-ray films of three infants who at autopsy had multiple cryptococcal granulomas. Three other cases of calcified torulomas are mentioned by Carton and Mount.¹ In a case reported by Liu,⁴ five separate "balls" of calcification about 1.2 cm in diameter were identified on skull films; at operation five calcified cryptococcal granulomas were removed. One other instance in a man with undiagnosed meningitis was briefly mentioned during a clinicopathological conference.⁵ At autopsy a toruloma near the sella turcica had healed with calcification and then had broken down to cause recurrent meningitis and death. Only one patient besides the one herein described has been reported as having a positive brain scan associated with a cryptococcal granuloma.⁶

Microbiologic diagnosis of cryptococcal meningo-encephalitis rests on culture of *Cryptococcus neoformans* from cerebrospinal fluid or direct visualization of its budding encapsulated yeast forms in the fluid by staining with india ink. Unfortunately, in recent years there have been numerous case reports documenting cryptococcosis of the central nervous system in which india ink preparations are negative (or misinterpreted)⁷ and lumbar sac cultures are sterile.⁸⁻¹⁰ This is amply demonstrated in the present case, in which on several occasions india ink smears and cultures on fungus media of large amounts (20 ml) of

spinal fluid concentrated by Millipore® filtration failed to grow the organism.

It has been suggested that if cryptococcal disease of the central nervous system is suspected, isolation of the fungus from sites outside the central nervous system may provide indirect evidence of associated neural involvement. Such sites include urine and lung.¹¹ Since the respiratory tract is the presumed portal of entry for the organism, unexplained lung nodules or diffuse infiltrates in appropriate patients should be examined for cryptococci. In this regard, transbronchial brush biopsy, a minimally invasive procedure, was recently used successfully for rapid identification of viable cryptococci in a pulmonary nodule.¹² Isolation of *Cryptococcus* from sputum alone may have less diagnostic significance since saprophytic colonization by the fungus without overt pulmonary disease has been demonstrated.^{13,14} Of interest is that the patient in the present case was noted to have a pulmonary coin lesion during his time in hospital in Hawaii. Unfortunately, the cause of this lesion was never clarified; it most likely represented the primary focus of cryptococcal infection.

Efforts have been made in the last several years to develop serologic test methods for cryptococcosis which would circumvent problems associated with detection and cultural isolation of the organism. Initial attempts were thwarted, however, by inconsistent demonstration of antibodies in patients with frank clinical disease. Recent investigation has concentrated instead on recognition of circulating cryptococcal polysaccharide antigen.¹⁵⁻¹⁷ In fact, presence of this antigen in serum and especially in spinal fluid has correlated best with active fungal infection. Failure to demonstrate diagnostic titers of antibody from similar specimens of body fluid is presumed to be due to neutralization (or complexing) of antibody by excess antigen.

Goodman et al⁹ reported three cases of india ink-negative and culture-negative chronic meningitis secondary to *Cryptococcus* which were diagnosable solely on the basis of positive cerebrospinal fluid latex agglutination tests for cryptococcal antigen. The initial diagnostic brain biopsy in the present case preceded, by eight days, receipt of the report that his cerebrospinal fluid and serum contained cryptococcal antigen; in retrospect, specific antifungal drug therapy might have been initiated without resorting to diagnostic craniotomy in view of this positive serologic test. Furthermore, in a number of cases efficacy of

antifungal chemotherapy has coincided with reduction of excess cryptococcal antigen levels.^{9,18} In the present case the cerebrospinal fluid antigen titer fell from 1:8 to 1:2 after 1,730 mg of amphotericin B. Antibody determinations by tube agglutination and indirect fluorescence were negative on both occasions.

No serologic test provides 100 percent diagnostic reliability and false-negative tests for cryptococcal antigen have been reported in culture-positive cryptococcosis.¹⁵⁻¹⁷ Roberts et al described a patient with intracerebral cryptococcal granulomas in whom no antigen was demonstrable in cerebrospinal fluid or serum.⁶ Serologic determinations were made, however, after therapy and the authors suggested that a negative test could have reflected eradication of infection. It is still reasonable to expect that when cryptococcal infection occurs as circumscribed intracerebral mass lesions, immunologic detection of antigen in remote sites (namely, lumbar sac cerebrospinal fluid or serum) would be significantly more productive than attempts to visualize or isolate fungus from similar sites.

Treatment of invasive cryptococcosis remains less than optimal. Although effectiveness of amphotericin B in reducing fatality in cryptococcal meningitis is not disputed, relapse requiring supplementary treatment courses with this toxic drug and neurologic residua are not uncommon.^{8,11} Moreover, after many years of experience with amphotericin B a "curative" total dose has not been established. Recommended total dosage ranges between 2 and 2.5 grams intravenously in adults,⁸ or about 35 mg per kilogram of body weight. Various modes of administration have been used in attempts to avert drug toxicity. One approach has been to administer generally lower doses (15 to 40 mg) of amphotericin B daily such that peak serum levels are at least twice those necessary for *in vitro* inhibition of fungal growth;¹⁹ another approach is to give higher doses (1 to 1.5 mg per kg of body weight) of amphotericin B on an every other day schedule.²⁰ Nevertheless, some degree of nephrotoxicity is an almost invariable accompaniment of dosage in this latter range.

None of these intravenous regimens, however, has attacked the problem of poor penetrance of amphotericin B into the central nervous system. This problem of drug diffusion was particularly critical in the present case because of gross cryptococcal abscess formation and resultant internal

hydrocephalus. Despite the exquisite sensitivity of his cryptococcal isolate to amphotericin B (minimum inhibitory concentration = 0.06 mcg per ml*), it was not unexpected that viable cryptococci were readily cultured from his surgically removed lesions while he was receiving large doses of the drug intravenously. Although amphotericin B has been given intrathecally (either in the lumbar sac or intraventricularly via an Ommaya subcutaneous reservoir²¹), there is insufficient clinical experience to evaluate its efficacy in the treatment of intracerebral torulomas.

A new orally administered anti-fungal compound, 5-fluorocytosine, may prove to be a rational alternative to amphotericin B in treatment of cryptococcal meningitis.^{6,22-24} Readily achievable levels of this drug in cerebrospinal fluid are much in excess of the minimum inhibitory concentration of most strains of *Cryptococcus neoformans*. An investigational supply of 5-fluorocytosine arrived at our hospital the day our patient died. Unfortunately the organism in this case was not especially susceptible to the drug (minimum inhibitory concentration = 6 mcg per ml with a minimum fungicidal concentration = 200 mcg per ml*). Since cryptococci exhibiting high level resistance (that is, greater than 1,000 mcg per ml) may emerge during therapy with 5-fluorocytosine,²⁴ it may not be prudent to employ the drug alone unless clearly fungicidal levels in cerebrospinal fluid can be achieved. In view of the relative *in vitro* resistance of our patient's cryptococcal isolate to 5-fluorocytosine, fungicidal concentrations of the drug in his spinal fluid would not have been attainable. There is, however, mounting evidence that combining 5-fluorocytosine with amphotericin B may be a more efficacious approach to anti-fungal chemotherapy.²³ Synergistic activity of this drug combination has been demonstrated *in vitro* against a variety of yeast-like organisms including *cryptococcus*,²⁵ and emergence of 5-fluorocytosine-resistant organisms in experimental murine cryptococcal meningitis has been reduced by simultaneous administration of the two drugs.²⁶ Nevertheless, controlled clinical trials utilizing such drug combinations in human disseminated cryptococcosis remain to be performed.

It is of interest to speculate whether any significance can be attached to isolation of *Erwinia* species from this patient's spinal fluid. Although

*Kindly performed in the laboratory of Dr. A. I. Braude, UC San Diego, utilizing a method described by Shadomy (Appl. Microb 17:871-877, 1969).

there have been some recent reports of septicemic and central nervous system infection in man caused by *Erwinia* species,^{27,28} many of the bacteremic episodes occurred in hospitals where intravenous infusion sets contaminated with the organism were used.^{29,30} Indeed, the *Erwinia* species was isolated from our patient during the peak of this epidemic of nosocomial bacteremias.

Summary

A case of multiple cryptococcal granulomas of the brain in a 47-year-old white man is described. The initial presentation was that of psychiatric illness. Unusual features of this illness were the presence of multiple intracranial calcifications as observed on skull x-ray films and foci of increased isotope uptake on brain scan. Multiple india ink preparations and fungus cultures of cerebrospinal fluid were negative. Diagnosis of cryptococcosis was not made until brain biopsy. The importance of serologic testing of both blood and spinal fluid for cryptococcal infection in patients with undiagnosed chronic meningitis or atypical cerebral space-occupying lesions is stressed.

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